

Scotland's Rural College

Tolerance and resistance to a nematode challenge are not always mutually exclusive

Athanasiadou, S; Tolossa, K; Debela, E; Tolera, A; Houdijk, JGM

Published in:

International Journal for Parasitology

DOI:

[10.1016/j.ijpara.2014.12.005](https://doi.org/10.1016/j.ijpara.2014.12.005)

Print publication: 01/01/2015

Document Version

Peer reviewed version

[Link to publication](#)

Citation for pulished version (APA):

Athanasiadou, S., Tolossa, K., Debela, E., Tolera, A., & Houdijk, JGM. (2015). Tolerance and resistance to a nematode challenge are not always mutually exclusive. *International Journal for Parasitology*, 45(4), 277 - 282. <https://doi.org/10.1016/j.ijpara.2014.12.005>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 **Tolerance and resistance to a nematode challenge are not always mutually exclusive**

2

3 Spiridoula Athanasiadou^{a*}, Ketema Tolossa^{a,b}, Etana Debela^c, Adugna Tolera^d, Jos GM Houdijk^a

4

5 ^aDisease Systems, SRUC, Easter Bush, EH25 9RG, United Kingdom. ^bDepartment of Chemistry,

6 College of Natural and Computational Science, Hawassa University, P.O. Box 05, Hawassa,

7 Ethiopia. ^cSchool of Veterinary Medicine, Hawassa University, P.O. Box 05, Hawassa,

8 Ethiopia. ^dSchool of Animal and Range Science, College of Agriculture, Hawassa University, P.O.

9 Box 222, Hawassa, Ethiopia.

10

11

12

13

14

15 *Corresponding author: spiridoula.athanasiadou@sruc.ac.uk

16 Animal and Veterinary Sciences, SRUC

17 Roslin Institute Building, Easter Bush, Midlothian, EH25 9RG

18 Scotland, UK

19 Telephone number: +44 131 6519355

20

21

22

23

24 Abstract

25 The relationship between the manifestations of tolerance (host's ability to reduce the impact of a
26 given level of pathogens) and resistance (host's ability to clear pathogens) has been assumed to be
27 an antagonistic one. Here we tested the hypothesis that mice from strains more resistant to
28 intestinal nematodes will experience reduced tolerance compared to less resistant mice. Three
29 inbred strains of mice were used: C57BL/6 mice have been characterised as susceptible, whereas
30 BALB/c and NIH mice have been characterised as resistant to *Heligmosomoides bakeri* infection.
31 Mice of each strain were either parasitized with a single dose of 250 L₃ *H. bakeri* (n=10) in water or
32 were sham-infected with water (n=10). Body weight, food intake and worm egg output were
33 recorded regularly throughout the experiment. Forty-two days post infection mice were
34 euthanized and organ weights, eggs in colon and worm counts were determined. C57BL/6 mice
35 showed significantly greater worm egg output ($P<0.001$), eggs in colon ($P<0.05$) and female worm
36 fecundity ($P<0.05$) compared to NIH and BALB/c. Parasitized BALB/c mice grew more whilst
37 parasitized C57BL/6 grew less than their sham-infected counterparts during the first two weeks
38 post challenge ($P=0.05$). Parasitism significantly increased liver, spleen, small intestine and
39 caecum weights ($P<0.001$), but reduced carcass weight ($P<0.01$). Average daily gain and worm
40 numbers were positively correlated in NIH mice ($P=0.05$); however, the relationship was reversed
41 when carcass weight was used as a measure for tolerance. BALB/c mice did not appear to suffer
42 from the consequences of parasitism, with carcass weight similar in all animals. Our hypothesis
43 that strains more resistant to the *H. bakeri* infection are less tolerant compared to less resistant
44 strains is rejected, as the two resistant strains showed variable tolerance. Thus, resistance and
45 tolerance to intestinal nematode infection are not always mutually exclusive.

46

47 Keywords: mice; genetic resistance; infection tolerance; nematodes; performance;

48 *Heligmosomoides bakeri*.

49

50 1. Introduction

51 The intestinal trichostrongyloid nematode *Heligmosomoides (polygyrus) bakeri* has been used as a
 52 model of chronic intestinal nematode infection for over four decades (Behnke et al., 2006). *H.*
 53 *bakeri* infection in mice induces a strongly polarized Th2 immune response, which has been shown
 54 to be critical for worm control and expulsion (Reynolds et al., 2012). The mechanisms
 55 underpinning helminth expulsion in mice are studied to facilitate predictions about the outcome
 56 of similar interactions between helminths and the immune system in livestock and humans, to
 57 enable the development of control strategies. The outcome of primary *H. bakeri* infection is
 58 strongly influenced by the genetic background of mice, with strains differing in their susceptibility
 59 to chronic infection (Reynolds et al., 2012). Whilst between-strain variation in nematode
 60 resistance has been previously described (Behnke et al., 2006), there is no evidence of description
 61 of variation in tolerance. Resistance describes the ability of the host to clear pathogens, whereas
 62 tolerance describes the ability to reduce the health or fitness impact of a given infection intensity
 63 (Ayres and Schneider, 2012; Raberg, 2014). Characterising the tolerance of mice strains that differ
 64 in their resistance to *H. bakeri* infection will facilitate the selection of the most appropriate mouse
 65 strain to model nematodiasis in human and livestock hosts.

66
 67 The study of tolerance to parasites and its association with resistance has a long tradition in plant
 68 science, but very limited evidence is available from animals. Recently, the individual variation in
 69 tolerance to parasites has been described, in wild sheep and rodent populations (Hayward et al,
 70 2014; Jackson et al, 2014). Haward et al (2014) observed a positive relationship between tolerance
 71 and evolutionary fitness in sheep, with more tolerant animals having higher lifetime breeding
 72 success. Jackson et al (2014) on the other hand observed a negative relationship between
 73 tolerance and reproduction, with more tolerant animals having reduced reproductive effort. The

74 association between the manifestations of tolerance and resistance to nematodes in animals has
75 been previously assumed to be an antagonistic one (Doeschl-Wilson et al., 2009), although there is
76 no experimental evidence to support this view. Råberg (2014) argued that when analysed
77 simultaneously, tolerance and resistance should be under correlational selection; this implies
78 either a negative relationship between these traits, or the possibility they may be both at
79 intermediate levels.

80

81 Here we tested the hypothesis that between-strain variation in resistance to *H. bakeri* infection
82 will correlate with between-strain variation in tolerance. The expectation was that mouse strains
83 more resistant to the *H.bakeri* infection will be less tolerant compared to less resistant strains.

84

85 **2. Materials and Methods**

86

87 *2.1. Experimental animals and housing*

88 This animal experiment was approved by SRUC's Ethical Review Committee (ED AE 06/2011) and
89 carried out under Home Office authorization (PPL 60/3626). A total of 60 5-wks-old male C57BL/6,
90 NIH and BALB/c mice (n=20 per strain), were housed in a room with an ambient temperature of
91 $21\pm1^{\circ}\text{C}$ and a 12 h light cycle (07.00 to 19.00 h). Mice were individually housed in solid bottomed
92 cages with fresh sawdust and bedding material provided weekly. Shredded paper was added as
93 environmental enrichment. The three strains were selected based on variation in their
94 susceptibility to infection with *H. bakeri* as defined by their phenotype during the infection.
95 C57BL/6 mice have been characterised as poor responders to *H. bakeri*; they maintain a high
96 worm burden that can persist for over 30 weeks (Behnke et al., 2006). NIH mice have been
97 characterised as strong, early responders to *H. bakeri*; compared to C57BL/6 mice, worm

establishment is lower, and worm burdens are cleared out within seven weeks (Behnke *et al.*, 2006). BALB/c mice have also been characterised as strong responders, although worm expulsion rate is slower than in NIH mice (Behnke *et al.*, 2006; Reynolds *et al.*, 2012).

2.2. Infection protocol and experimental design

The experiment was conducted over two consecutive blocks, balanced for all treatments, of 30 mice each. At day 0 of the experiment, mice of each strain received either a single dose of 250 *H. bakeri* L₃ suspended in 0.2 ml of water (n=10) or a sham infection of 0.2 ml of water (n=10) via oral gavage (Houdijk and Bunger, 2007). The *H. bakeri*, formerly known as *Heligmosomoides polygyrus bakeri* and *Nematospiroides dubius* (Cable *et al.*, 2006), were cultured from mono-specifically infected donor mice. The dose of *H. bakeri* was chosen to produce a subclinical level of infection that has been shown to affect mice growth (Houdijk and Bunger, 2006; 2007).

Mice were fed *ad libitum* throughout the experiment a maintenance diet (14% crude protein; Special Diet Services, Lillico Biotechnologies, UK). Mice were monitored for 42 days post nematode infection. On day 42 they were euthanized for sample recovery.

2.3. Measurements and sample collection

Body weight and food intake: Mice and food refusals were weighed three times weekly throughout the experiment. On each of these days food refusals were weighed out and fresh food added weighed in. From these measurements, food intake was calculated per mouse per day. Food intake and body weight of mice were used as indicators of growth performance.

121 Nematode egg counts: Mice were placed onto wire-bottomed cages and faecal samples were
 122 collected on wetted cardboard on days 17, 24, 31, 38 post infection to assess faecal egg counts
 123 (eggs per g faeces). This was carried out using a modified flotation technique (Christie and Jackson,
 124 1982). Faeces were collected over a 12h period, during which a constant rate of egg production
 125 was assumed. Egg output was expressed as eggs per 12h (EO) to account for possible dilution
 126 effect on faecal egg counts attributed to variable faecal outputs as a consequence of different
 127 food intake in different mice strains (Coltherd et al., 2009).

128

129 Internal organ weights, eggs in colon and worm burdens: On day 42 mice were humanely killed
 130 via CO₂ inhalation and dissected for sample recovery. The small intestine was weighed, opened up
 131 and placed in a tube with PBS, which was then incubated at 37°C for 3 h to allow worms to migrate
 132 out of the tissue. Tissue and recovered worms were stored in a 5% formalin solution. Male and
 133 female worms were separated and counted. The colon contents were weighed and an egg count
 134 was performed with the same floatation technique (Christie and Jackson 1982). The colon egg
 135 count was then multiplied by colon contents weight to account for dilution effects arising from
 136 variation in food intake and colon content volumes between the different strains. Resultant data
 137 were expressed as number of eggs in colon (EIC, number of eggs). The EIC was divided by the
 138 number of females counted to obtain an estimate for the *per capita* fecundity (eggs per female).
 139 EO, EIC, *per capita* fecundity and total worm counts were used to confirm variation in resistance of
 140 the strains. EIC and worm counts were used in tolerance estimates, as explained below.

141 Measures of tolerance: Individual tolerance was estimated in two different ways. Firstly, we
 142 associated carcass weight at dissection (true reflection of performance) and worm burdens
 143 recovered at dissection (accurate estimate of parasite load). We also associated average daily gain,
 144 which is often used as an indirect indicator for performance and eggs in colon (EIC), as an indirect

145 indicator for parasite load. Our expectation was that tolerance estimates will be similar in both
 146 cases.

147

148 2.4. Statistical analysis

149 Data were analysed in Genstat 11th Edition (VSN International LTD, 2008). Model assumptions
 150 were tested on normality of means and residuals. Average daily body weight gain and food intake
 151 during worm establishment (P1: days 0-16) and the established infection (P2: days 17-42) were
 152 analysed through a 3 x 2 factorial ANOVA (3 strains x 2 levels of parasitism) with pre-infection
 153 body weight used as covariate. Carcass and internal organ weights were analysed through the
 154 same 3 x 2 factorial ANOVA, again with pre-infection body weight as covariate. EIC, EO, worm
 155 counts and worm fecundity were analysed in parasitised animals by one-way ANOVA (3 strains).
 156 The EO data were analysed in a repeated measure model. Due to the skewed nature of the data,
 157 EO, EIC and *per capita* fecundity were log₁₀ (n) transformed. These are reported as back-
 158 transformed least-square means, accompanied by lower and upper confidence intervals,
 159 calculated from back-transforming least-square mean of transformed data (m), m–S.E. and m+S.E
 160 respectively. Experimental round was included in both statistical models as block. Effects with P-
 161 values less than 0.05 are considered significant whilst those with P-values between 0.05 and 0.10
 162 are described as tendencies or trends. Pearson's correlations were performed between
 163 aforementioned *a priori* selected parasitological and performance data to characterise the
 164 tolerance of different mice strains to the *H. bakeri* infection.

165

166 3. Results

167 3.1. *H. bakeri* nematodes were more prolific in C57BL/6 than in NIH or BALB/c mice

168 There was a significant strain effect on EO ($P<0.001$) with C57BL/6 mice excreting more eggs
 169 compared to NIH and BALB/c. EO eggs/12h reached 48,000 (39,000-60,000) in C57BL/6 mice,
 170 25,000 (20,000-31,000) in BALB/c mice and 24,000 (20,000-30,000) in NIH mice, day 38 post
 171 challenge. There was a significant time effect, with EO increasing over time in all strains ($P=0.004$).
 172 The strain x time interaction was also significant, with the rate of increase being greater in
 173 C57BL/6 mice compared to mice from the other strains (Figure 1).

174

175 <<Figure 1 here>>

176

177 EIC determined on day 42 was significantly affected by strain at the same direction as EO
 178 ($P=0.033$); C57BL/6 mice had significantly greater EIC compared to mice of the other two strains
 179 (Figure 2).

180

181 <<Figure 2 here>>

182

183 There was no significant strain effect on total ($P=0.189$), male ($P=0.239$) or female ($P=0.156$) worm
 184 counts, although worm burdens were 40% greater in C57BL/6 mice compared to NIH (Figure 3).
 185 Strain significantly affected *per capita* fecundity ($P=0.045$), which averaged 535 (475-604), 338
 186 (275-415) and 280 (230-341) eggs/female worm in C57BL/6, BALB/c and NIH mice, respectively.

187

188 <<Figure 3 here>>

189

190 *3.2. The impact of parasitism on performance was short-lived and strain-dependant*

191 Infection reduced food intake in each strain during the first 16 days post challenge (P1); across
 192 strains, food intake of parasitized and sham-infected mice averaged 4.23 and 4.64 g/day,
 193 respectively ($P<0.001$; Table 1). NIH mice consumed the most and C57BL/6 consumed the least
 194 food ($P<0.001$). Between days 17-42 post infection (P2), the strain effect on food intake was
 195 sustained with C57BL/6 mice consistently eating the least and NIH mice eating the most food
 196 ($P<0.001$). However, the effect of parasitism disappeared during this period; across strains, food
 197 intake of parasitized and sham-infected mice averaged 4.40 and 4.32 g/day, respectively.

198

199 <<Table 1 here>>

200

201 During P1, body weight gain did not differ between parasitized and sham-infected mice across
 202 strains ($P=0.540$), whilst NIH mice grew faster than C57BL/6 mice, with BALB/c being intermediate
 203 ($P<0.001$; Table 1). However, an interaction demonstrated that parasitized BALB/c mice grew
 204 more and parasitized C57BL/6 grew less than their sham-infected counterparts ($P=0.056$). During
 205 P2, there was no significant difference in body weight gain between parasitized and sham-infected
 206 mice ($P=0.432$). The strain effect on body weight gain remained significant, with NIH mice growing
 207 faster compared to C57BL/6 and BALB/c mice ($P<0.001$).

208

209 *3.3. Parasitism and mouse strain had an impact on internal organs weight*

210 Strain significantly affected the weight of most internal organs measured (Table 2). The weight of
 211 liver, spleen, small intestine and caecum were significantly greater in parasitized mice compared
 212 to non-parasitized ones ($P<0.01$). However, the interaction between parasitism and strain was
 213 significant for caecum and spleen weight; the caecum weighed significantly more in parasitized
 214 C57BL/6 mice ($P=0.030$) compared to parasitized mice from the other strains, whereas the spleen

215 of parasitized NIH mice weighed significantly more compared to parasitized mice from the other
 216 strains ($P < 0.001$). Across strains, spleen weight was negatively correlated with total number of
 217 worms recovered and with EIC; mice with heavier spleens had fewer worms ($r = -0.46$; $P = 0.01$) and
 218 EIC ($r = -0.63$; $P = 0.001$)

219

220 Final body weight at dissection was affected by strain; NIH mice weighed the most and C57BL/6
 221 the least, with BALB/c weighing intermediate ($P = 0.028$). Carcass weight followed a similar pattern
 222 across strains ($P < 0.001$). Parasitism did not affect the final BW of mice but significantly reduced
 223 carcass weight ($P = 0.003$).

224

225 <<Table 2 here>>

226 <<Table 3 here>>

227

228 *3.4. Using body weight gain to estimate tolerance may underestimate the impact of parasitism on*
 229 *performance*

230 Table 3 shows that for NIH mice only, there was a significant positive correlation between
 231 averaged daily gain (ADG) and worm numbers ($r = 0.62$; $P = 0.05$). However, when carcass weight
 232 was used to calculate tolerance, this relationship was reversed; the greater the number of worms
 233 recovered at dissections, the lower the carcass weight of NIH mice ($r = -0.48$; $P > 0.05$). Although the
 234 correlations between carcass weight and worm counts in BALB/c mice were not significant, BALB/c
 235 mice appear to be the least affected by the infection compared to the other strains.

236

237 **4. Discussion**

238 This is the first study, where the tolerance to a nematode infection was quantified in strains of
239 mice that differ in their degree of resistance to *H. bakeri*. Three major outcomes were delivered
240 from the study. Firstly, we demonstrated that resistance and tolerance to a nematode parasite
241 infection are not necessarily mutually exclusive. The resistant BALB/c mice appear to be more
242 tolerant and least affected by the infection compared to the other strains. Secondly, we
243 demonstrated variation in tolerance between different strains of mice, which emphasizes the
244 importance of selecting the appropriate strain as a model of chronic nematode infection in
245 different mammalian hosts. Thirdly, we have clearly shown that body weight gain may not be the
246 best performance indicator to estimate the tolerance to parasitic challenge. Carcass
247 measurements revealed that the impact of parasitism was underestimated when based on body
248 weight gain measurement, as a consequence of an increase in the weight of internal organs in
249 parasitized mice, including spleen, liver, small intestine and caecum.

250

251 We used four measurements, namely EO, EIC, *per capita* fecundity and total worm counts to
252 confirm variation in resistance, i.e. the ability of the host to clear pathogens, of three mice strains.
253 Throughout the experiment, C57BL/6 mice excreted the largest number of eggs, compared to mice
254 from the other strains. Similarly, estimates of EIC and fecundity at dissection confirmed that
255 C57BL/6 mice were the most susceptible to the infection, as previously reported (Behnke et al,
256 2006). The genetic factors controlling resistance to *H. bakeri* include the major histocompatibility
257 complex (MHC) H-2 loci with C57BL/6 mice categorised as susceptible and BALB/c and NIH mice as
258 resistant genotypes (Reynolds et al, 2012). During trickle infections, differences in worm burdens
259 are evident within 6 weeks of the first challenge (Benkhe et al, 2006). However, following a single
260 challenge worm expulsion starts after week 10 post infections (Robinson et al, 1989), which would

261 be consistent with the similar worm numbers at 6 weeks post infection in all mice strains in our
262 experiment.

263

264 To estimate tolerance in the three strains of mice, body weight throughout the experiment and
265 carcass at dissections were taken. This detailed monitoring revealed that using carcass weight to
266 estimate tolerance may be the most appropriate indicator of animal performance. Parasitism
267 reduced carcass weight by an average of 5%, an effect that was not evident from body weight
268 monitoring. This was due to increases in internal organ weight in parasitized mice, namely the
269 small intestine, caecum, liver and spleen, which masked the penalties of parasitism on
270 performance. Body weight gain measurements alone showed that parasitism did not penalize
271 performance of NIH and C57BL/6 mice, whereas parasitized BALB/c mice appeared to grow faster
272 than their sham-infected counterparts. Our findings strongly suggest that the well established
273 negative impact of gastrointestinal nematode infections on animal performance, as measured by
274 change in body weight (e.g. Sykes 1997) may be underestimated.

275

276 The increase in the weight of the small intestine observed in parasitized mice is in agreement with
277 previous studies (Wong et al., 2007). This may be related to increased local inflammatory
278 responses (Cywiniska et al 2004), plasma extravasation and mucin production as a consequence of
279 parasitism (Wakelin, 1978). Furthermore, crypt hypertrophy and an increase in villi height has
280 been observed in mice infected with *H. polygyrus*; it has been hypothesised that this response is
281 adaptive to increase mucosal surface, to maintain nutrient absorption in the presence of the
282 nematodes (Wong et al., 2007). Spleen size was significantly increased in parasitized mice; spleen
283 enlargement has been observed during infections with filarial and gastrointestinal nematodes in
284 variety of hosts (John, 1994; Wong et al., 2007). This increase in size is likely the direct outcome of

splenic cell proliferation (Katona et al., 1983), which reflects host resistance. Although spleen size was increased in all parasitized animals, the increase was greater in the resistant NIH mice, which is in agreement with previous evidence (Ali and Behnke, 1985). *H. bakeri* infection also affected liver weight in all strains. Hepatomegaly has been previously associated with nematode infection, particularly during the visceral migration of the larvae in species such as *Toxocara canis* (Pecinali et al., 2005) and in certain strains of mice during *H. bakeri* infection (Wong et al., 2007). It has been shown that seven days post a *H. polygyrus* infection liver cytokines, including pro-inflammatory cytokines, were up-regulated, in the absence of pathological and inflammatory response (Helmby, 2009). This early up-regulation of pro-inflammatory cytokines, such as IFN- γ , may be responsible for a liver inflammation at later stages of infection, which may result in hepatomegaly, as this was observed in our study.

296

Our hypothesis was that strains more resistant to the *H. bakeri* infection are less tolerant compared to less resistant strains. As a consequence of the detailed monitoring of host's performance, it became apparent that the two resistant strains varied in their tolerance levels (Table 3), with BALB/c more tolerant than NIH mice and thus the hypothesis is rejected. This supports the view that disease resistance and tolerance are not always mutually exclusive, but they may both be at intermediate levels if this promotes evolutionary fitness (Råberg, 2014). The mechanistic basis of this relationship is still to be determined. The study has also clearly characterised the variation in tolerance to *H. bakeri* of different strains of mice. Although it is unclear at this stage what the underlying basis of this variation was, it ought to be considered to inform on model choice for chronic nematode infection in human and animal hosts. BALB/c mice do not appear to be a good model for livestock, as livestock do not tolerate well nematode challenge, which is evident from the penalties on performance within weeks from the onset of an

infection (Coop et al, 1982). However, in humans, nematode parasitic gastroenteritis may be asymptomatic and is often not manifested with changes in body weight or body weight gain (<http://emedicine.medscape.com/article/224011-clinical>). Therefore, BALB/c mice may be a more appropriate model for parasitic gastroenteritis in humans, whilst C57BL/6 mice may be the better model for livestock.

314

315

316 **Acknowledgements**

This work was supported by the UK Biotechnology and Biological Sciences Research Council (BBRSC), Department for International Development (DFID) and Scottish Government (SG) under the umbrella of their CIDLID initiative (BB/H009299/1). We are thankful to Julie van Mierlo, Thom van Overbeek and Sandra Terry for their technical support. We are also thankful to Ian Nevison from Biomathematics and Statistics Scotland for statistical advice.

322

323 **References**

324

- Ali, N.M.H., Behnke, J.M., 1985. Observations on the gross changes in the secondary lymphoid organs of mice infected with *Nematospiroides dubius*. J. Helminthol. 59, 167- 174.
- Ayres, J.S., Schneider, D.S., 2012. Tolerance of Infections. Annu. Rev. Immunol. 30, 271–294.
- Behnke, J. M., Mugambi, J. M., Clifford, S., Iraqi, F. A., Baker, R. L., Gibson, J. P., Wakelin, D., 2006. Genetic variation in resistance to repeated infections with *Heligmosomoides polygyrus bakeri*, in inbred mouse strains selected for the mouse genome project. Parasite Immunol. 28, 85-94.

- 332 Cable, J., Harris, P.D., Lewis, J.W., Behnke, J.M., 2006. Molecular evidence that *Heligmosomoides*
 333 *polygyrus* from laboratory mice and wood mice are separate species. *Parasitology* 133, 111–
 334 122.
- 335 Christie, M., Jackson, F., 1982. Specific identification of Strongyle eggs in small samples of sheep
 336 faeces. *Res. Vet. Sci.* 32, 113–117.
- 337 Coltherd, J.C., Bünger, L., Kyriazakis, I., Houdijk, J.G.M., 2009. Genetic growth potential interacts
 338 with nutrition on the ability of mice to cope with *Heligmosomoides bakeri* infection.
 339 *Parasitology* 136, 1043-1055.
- 340 Coop, R.L., Sykes, A.R., Angus, K.W., 1982. The effect of three levels of intake of *Ostertagia*
 341 *circumcincta* larvae on growth rate, food intake and body composition of growing lambs. *J.*
 342 *Agric. Sci.* 98, 247–255.
- 343 Cywińska, A., Czumińska, K., Schollenberger, A., 2004. Granulomatous inflammation during
 344 *Heligmosomoides polygyrus* primary infections in FVB mice. *J. Helminthol.* 78, 17-24.
- 345 Doeschl-Wilson, A.B., Brindle, W., Emmans, G., Kyriazakis, I., 2009. Unravelling the relationship
 346 between animal growth and immune response during micro-parasitic infections. *PLoS One.* 19,
 347 e7508. doi: 10.1371/journal.pone.0007508.
- 348 Hayward, A.D., Nussey, D.H., Wilson, A.J., Berenos, C., Pilkington, J.G., Watt, K.A., Pemberton, J.M.,
 349 Graham, A.L., 2014. Natural selection on individual variation in tolerance of gastrointestinal
 350 nematode infection. *PLoS Biology* 12 (7), e1001947
- 351 Helmby, H., 2009. Gastrointestinal nematode infection *J. Immunol.* 182, 5663-5671.
- 352 Houdijk, J.G.M., Bünger, L., 2006. Selection for growth increases the penalty of parasitism on
 353 growth performance in mice. *Proc Nut Soc* 65, 68A.

- 354 Houdijk, J. G. M., Bünger, L., 2007. Interactive effects of selection for growth and protein supply on
 355 the consequences of gastrointestinal parasitism on growth performance in mice. Proc Brit Soc
 356 Anim Sci, pp 92.
- 357 Jackson, J.A., Hall, A.J., Ralli, C., Lowe, A., Zawadzka, M., Turner, A.K., Stewart, A., Birtles, R.J.,
 358 Paterson, S., Brsdlay, J.E., Begon, M., 2014. An immunological marker of tolerance to infection
 359 in wild rodents. PLoS Biology, 12(7), e1001901.
- 360 John, J.L., 1994. Nematodes and the spleen: an immunological relationship. Experientia (Basel), 50,
 361 15–22.
- 362 Katona, I.M., Urban, J.F. Jr, Scher, I., Kanellopoulos-Langevin, C., Finkelman, F.D. 1983. Induction of
 363 an IgE response in mice by *Nippostrongylus brasiliensis*: characterization of lymphoid cells with
 364 intracytoplasmic or surface IgE. J. Immunol. 130, 350-356.
- 365 Pecinali, N.R., Gomes, R.N., Amendoeira, F.C., Pereira Bastos, A.C.M., Martins, M.J.Q.A., Pegado,
 366 C.S., Pereira Bastos, O.M., Bozza, P.T., Castro-Faria-Neto, H.C., 2005. Influence of murine
 367 *Toxocara canis* infection on plasma and bronchoalveolar lavage fluid eosinophil numbers and its
 368 correlation with cytokine levels. Vet. Parasitol. 134, 121-130.
- 369 Raberg, L., 2014. How to live with the enemy: understanding tolerance to parasites. PLoS Biology,
 370 12 (11), e1001989.
- 371 Reynolds, L.A., Filbey, K.J., Maizels, R.M., 2012. Immunity to the model intestinal helminth parasite
 372 *Heligmosomoides polygyrus*. Seminars in Immunopathology 34, 829-846.
- 373 Robinson, M., Wahid, F., Behnke, J.M., Gilbert, F.S., 1989. Immunological relationships during
 374 primary infection with *Heligmosomoides polygyrus* (*Nematospiroides dubius*): dose-dependent
 375 expulsion of adult worms. Parasitology 98, 115-124.

- 376 Sykes, A.R., 1997. Effects of nematode parasitism on ruminant performance. In: Sustainable
377 Control of Internal Parasites in Ruminants: Animal Industries Workshop, Barrell GK (ed),
378 Canterbury, New Zealand, Lincoln University, pp. 81–92.
- 379 Wakelin, D., 1978. Genetic control of susceptibility and resistance to parasitic infection. Adv.
380 Parasitol. 16 , 219–308.
- 381 Wong, T., Hildebrandt, M., Thrasher, S.M., Appleton, J.A., Ahima, R.S., Wu, G.D., 2007. Divergent
382 metabolic adaptations to intestinal parasitic nematode infection in mice susceptible or resistant
383 to obesity. Gastroenterology. 133, 1979–1988.
- 384
- 385

386 **Legends to Figures**

387

388 Figure 1. Backtransformed means of egg output (EO) of mice infected with 250 *Heligmosomoides*
 389 *bakeri* infective larvae. Mice were one of three strains (n=10): C57BL/6, NIH, or BALB/c, which
 390 have been characterised as susceptible or resistant to *H. bakeri* infection. Lower and upper error
 391 bar values are backtransformed values of log-transformed mean minus or plus its standard error,
 392 respectively.

393

394 Figure 2. Backtransformed means of colon egg counts (CEC) of mice infected with 250
 395 *Heligmosomoides bakeri* infective larvae. Mice were one of three strains (n=10): C57BL/6, NIH, or
 396 BALB/c, which have been characterised as susceptible or resistant to *H. bakeri* infection. Lower
 397 and upper error bar values are backtransformed values of log-transformed mean minus or plus its
 398 standard error, respectively.

399

400 Figure 3. Backtransformed means of adult male, adult female, and total worm burdens of mice
 401 infected with 250 *Heligmosomoides bakeri* infective larvae. Mice were one of three strains (n=10):
 402 C57BL/6, NIH, or BALB/c, which have been characterised as susceptible or resistant to *H.bakeri*
 403 infection. Lower and upper error bar values are backtransformed values of log-transformed mean
 404 minus or plus its standard error, respectively.

Figure 1

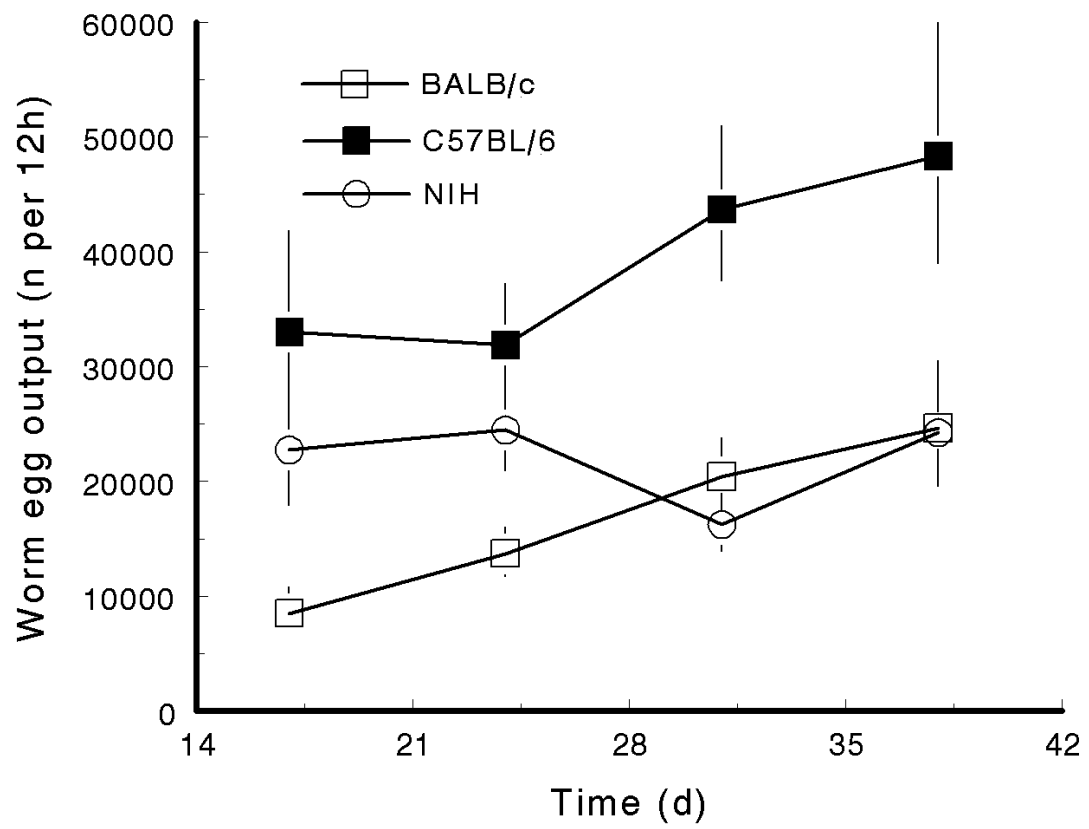


Figure 2

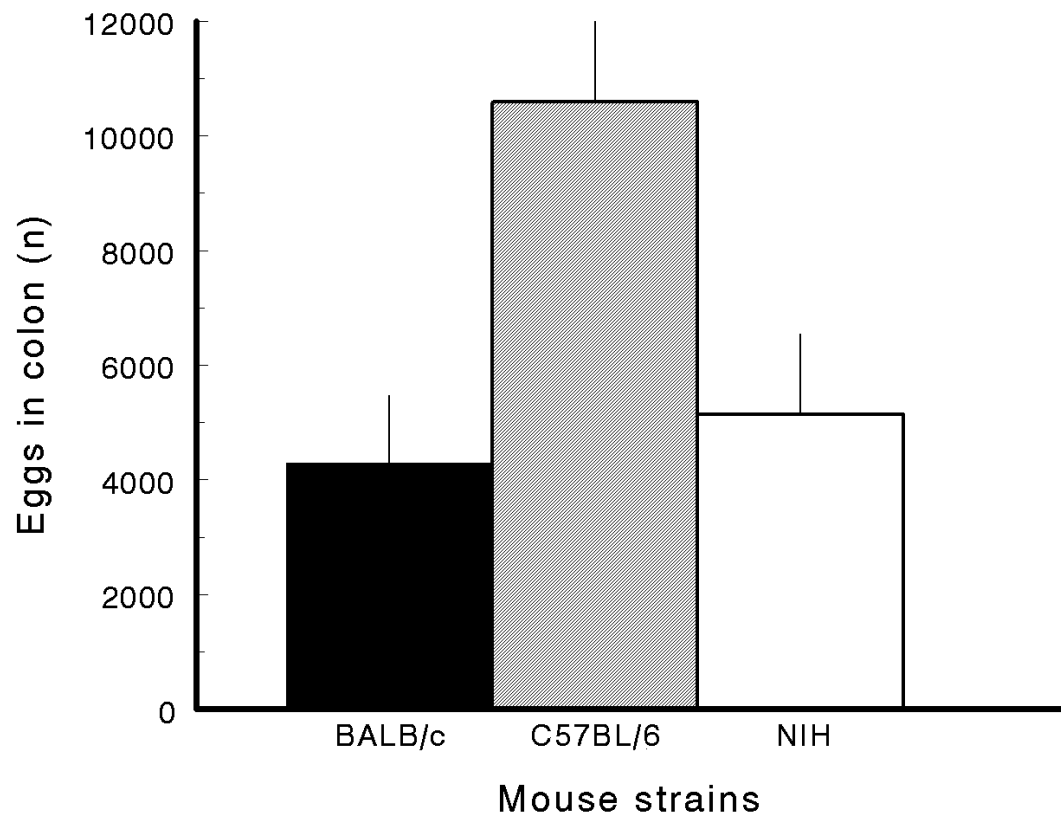


Figure 3

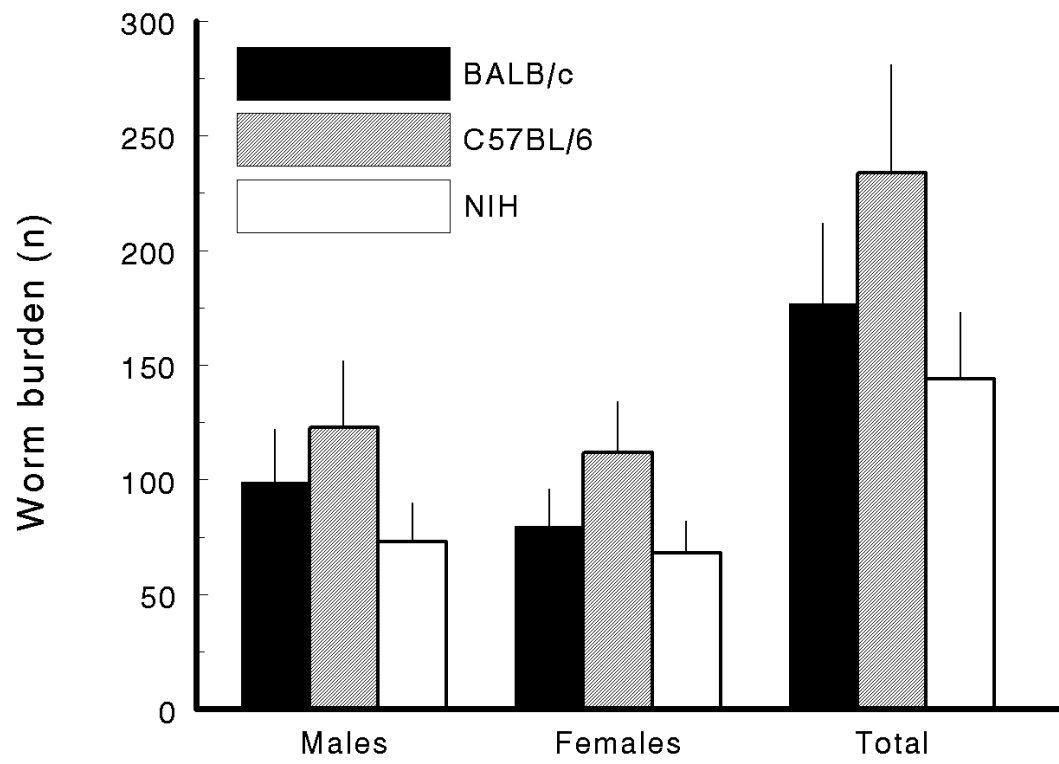


Table 1. Mean voluntary food intake (VFI, g/day) and average daily gain (ADG, g/day) of C57BL/6, NIH or BALB/c mice over 42 days after infection with 250 *Heligmosomoides bakeri* infective larvae in 0.2 ml water (Par) or sham-infected with 0.2 ml water (Sham).

Strain	Treatment	Period 1 ¹		Period 2 ¹	
		VFI	ADG	VFI	ADG
BALB/c	Par	4.18	0.271	4.52	0.152
	Sham	4.56	0.187	4.46	0.115
C57BL/6	Par	3.84	0.130	3.71	0.097
	Sham	4.01	0.169	3.69	0.117
NIH	Par	4.84	0.359	4.97	0.157
	Sham	5.31	0.365	4.80	0.151
	S.E.D. ²	0.095	0.036	0.114	0.021
Significance (P values) ³					
Strain		<0.001	<0.001	<0.001	0.009
Parasitism		<0.001	0.540	0.149	0.432
Strain × Parasitism		0.179	0.056	0.579	0.126

¹Period 1 relates to nematode establishment (day 0 – 16), whereas Period 2 relates to having a potent infection (day 1-42)

²Standard error of the differences between the means for the interaction term (n=10)

³The effect of the block was not significant and thus results are reported across the two blocks

Table 2. Final body weight, carcass, viscera and spleen weights (g) of C57BL/6, NIH or BALB/c mice, 42 days after infection with 250 *Heligmosomoides bakeri* infective larvae in 0.2 ml water (Par) or sham-infected with 0.2 ml water (Sham).

Strain	Treatment	BW ¹	Carcass	Stomach	SI ²	LI ³	Caecum	Liver	Spleen
BALB/c	Par	29.1	21.5	0.34	2.83	0.35	0.68	1.50	0.138
	Sham	27.1	21.7	0.39	1.59	0.32	0.51	1.32	0.101
C57BL/6	Par	25.3	18.9	0.39	2.73	0.24	0.92	1.23	0.097
	Sham	25.4	20.8	0.32	1.45	0.21	0.59	1.11	0.067
NIH	Par	32.6	24.8	0.57	3.07	0.32	0.68	1.71	0.195
	Sham	32.3	26.2	0.48	1.93	0.29	0.63	1.58	0.113
	S.E.D. ⁴	0.90	0.68	0.093	0.121	0.040	0.073	0.075	0.006
Significance (P-values) ⁵									
Strain		<0.001	<0.001	0.022	<0.001	0.002	0.020	<0.001	<0.001
Parasitism		0.166	0.003	0.475	<0.001	0.258	<0.001	0.002	<0.001
Strain × Parasitism		0.214	0.168	0.503	0.679	0.966	0.030	0.792	<0.001

¹Final body weight at post mortems

²Small Intestine with content

³Large Intestine without content

⁴Standard error of the differences between the means for the interaction term (n=10)

⁵The effect of the block was not significant and thus results are reported across the two blocks.

Table 3. Pearson's correlations¹ between worm counts or the number of worm eggs in the colon contents (EIC), and average daily body weight gain (ADG), carcass and spleen weight in selected mice strains (n=10).

		BALB/c	C57BL/6	NIH
Worms	ADG	0.22	0.12	0.62*
	Carcass	0.37	-0.08	-0.48
	Spleen	-0.35	-0.62*	-0.49
EIC	ADG	0.30	-0.31	0.33
	Carcass	0.50	-0.06	-0.15
	Spleen	0.16	-0.36	-0.50

¹Superscripts denote significance of r at P<0.05 (*)